UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: March 29, 2012

SUBJECT: Tetramethrin: Immuotoxicity study in rats

PC Code: 069003
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0052898
MRID No.: 48540401

DP Barcode: 392709
Registration No.: GDCI-069003-28621
Regulatory Action: N/A
Submission No.: N/A
CAS No.: 7696-12-0
40 CFR: N/A

FROM: **Yung G. Yang, Ph.D.**
Risk Assessment Branch VI
Health Effects Division (7509 P)

THROUGH: **Felecia Fort, Chief**
Risk Assessment Branch VI
Health Effects Division (7509 P)

TO: **Elissa Reaves, Chief**
Risk Assessment Branch IV
Health Effects Division (7509P)
And
Monica Wait
RMIB3
Pesticide Re-evaluation Division (7508P)

I. CONCLUSIONS

The immunotoxicity study in rats for Tetramethrin (MRID 48540401) has been reviewed and classified as acceptable/guideline and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800).

Rec'd 4.2.12 1 of 14

II. BACKGROUND and ACTION REQUESTED

An immunotoxicity study on Tetramethrin (MRID 48540401) was submitted to address one of the data requirements for RED DCI. RAB VI was asked to review and prepare a DER for this study.

III. RESULTS AND DISCUSSION

The immunotoxicity study in rats for Tetramethrin (MRID 48540401) has been reviewed. The DER is attached and an executive summary is as follows:

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48540401), Tetramethrin (95.0% a.i., Batch No. 091007) was administered in the diet to female Sprague-Dawley rats (10/group) at concentrations of 0 (control), 1000, 2500, or 5000 ppm (equivalent to 0, 102, 257 or 545 mg/kg/day, respectively) for 28 consecutive days. Animals in the positive control group (8/group) received a single dose of cyclophosphamide (50 mg/kg) by intraperitoneal injection on Day 27. Four days before necropsy, animals in all groups were immunized with a suspension of sheep red blood cells ($2x10^8$ SRBC/animal) by intravenous injection (1.0 mL/animal). On Day 29, animals were sacrificed by carbon dioxide asphyxiation and exsanguination. Spleens were harvested for evaluation of T-cell dependent antibody response (TDAR) with a Jerne antibody-plaque forming cells (PFC) assay. Other parameters evaluated were: clinical observations, mortality, body weight, body weight gain, food and water consumption, select organs (adrenals, lymph nodes (mandibular, mesenteric, and left axillary), Peyer's patches, spleen, and thymus), organ weights (i.e. adrenals, spleen and thymus), and gross pathology investigations.

There were no treatment-related effects on mortality, clinical observations, food or water consumption, mean absolute and relative (to terminal body weight) organ (adrenals, spleen, or thymus) weights in any group.

There was a statistically significant reduction of body weight gain in females receiving 2500 or 5000 ppm (↓32% and ↓48%, respectively for Days 1-8). Mean body weight gains remained low in animals receiving 5000 ppm in the diet for the remaining treatment period. The overall (Day 1-29) body weight gains were statistically significantly decreased in the 2500 and 5000 ppm groups (↓15% and ↓24% respectively) compared to controls.

For systemic toxicity related to treatment with Tetramethrin, the LOAEL was 2500 ppm (545 mg/kg/day); based on overall decreased mean body weights gains. The NOAEL for systemic toxicity in female Sprague-Dawley rats is 1500 ppm (257 mg/kg/day).

Evaluation of immune function using an antibody plaque-forming cell (PFC) assay indicated that Tetramethrin had no treatment-related effect on specific activity (PFC/10^6 cells), total activity (PFC/spleen), cellularity/spleen, or spleen and thymus weights. High inter-individual variability was seen in the treatment and vehicle control groups.

Examination of individual animal data did not show any distribution that would demonstrate significant suppression of the anti-SRBC immune function parameters. The positive control group showed statistically significant decreases in immune function parameters, confirming the validity of the immunotoxicity assay.

The Natural Killer (NK) cells activity was not evaluated in this study. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

For immunotoxicity related to treatment with Tetramethrin, the NOAEL is 5000 ppm (equivalent to 545 mg/kg/day, the highest dose tested). The LOAEL was not determined (>5000 ppm).

This 28-day dietary immunotoxicity study in the rat is **acceptable/guideline** and satisfies guideline requirement for an immunotoxicity study (OPPTS 870.7800).

DATA EVALUATION RECORD
Tetramethrin
PC Code: 069003
TXR#: 0052898
MRID#: 48540401

Study Type: Immunotoxicity - Rats
OPPTS 870.7800

Prepared for

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Office of Pesticide Programs
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Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task Number: 1-1-79
EPA Reviewer//WAM: Yung Yang//Brunsman/Farwell

Disclaimer

This review may have been altered by the EPA subsequent to the contractors' signature above.

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** 3/28/2012
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Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 3/28/12
Template version 09/11

TXR#: 0052898

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dietary Immunotoxicity - Rat
OPPTS 870.7800

PC CODE: 069003 **DP BARCODE:** D392709

TEST MATERIAL (PURITY): Tetramethrin (95.0% a.i.)

SYNONYMS: Neopynamin; **IUPAC:** cyclohex-1-ene-1,2-dicarboximidomethyl (1RS,3RS;1RS,3SR)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate or cyclohex-1-ene-1,2-dicarboximidomethyl (1RS)-cis-trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate or cyclohex-1-ene-1,2-dicarboximidomethyl (±)-cis-trans-chrysanthemate

CITATION: Chesher, C.J. (2011) Tetramethrin: 4 Week Dietary Immunotoxicity Study in the Female Sprague-Dawley Rat. Huntingdon Life Sciences Ltd., (Huntingdon, Cambridgeshire, PE28 4HS, England). Huntingdon Life Sciences Project ID: VRY0022, July 14, 2011. MRID 48540401. Unpublished.

SPONSOR: Sumitomo Chemical Co. Ltd, 27-1, Shinkawa 2-chome, Chuo-ku, Tokyo 104-8260, Japan

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48540401), Tetramethrin (95.0% a.i., Batch No. 091007) was administered in the diet to female Sprague-Dawley rats (10/group) at concentrations of 0 (control), 1000, 2500, or 5000 ppm (equivalent to 0, 102, 257 or 545 mg/kg/day, respectively) for 28 consecutive days. Animals in the positive control group (8/group) received a single dose of cyclophosphamide (50 mg/kg) by intraperitoneal injection on Day 27. Four days before necropsy, animals in all groups were immunized with a suspension of sheep red blood cells ($2x10^8$ SRBC/animal) by intravenous injection (1.0 mL/animal). On Day 29, animals were sacrificed by carbon dioxide asphyxiation and exsanguination. Spleens were harvested for evaluation of T-cell dependent antibody response (TDAR) with a Jerne antibody-plaque forming cells (PFC) assay. Other parameters evaluated were: clinical observations, mortality, body weight, body weight gain, food and water consumption, select organs (adrenals, lymph nodes (mandibular, mesenteric, and left axillary), Peyer's patches, spleen, and thymus), organ weights (i.e. adrenals, spleen and thymus), and gross pathology investigations.

There were no treatment-related effects on mortality, clinical observations, food or water consumption, mean absolute and relative (to terminal body weight) organ (adrenals, spleen, or thymus) weights in any group.

There was a statistically significant reduction of body weight gain in females receiving 2500 or 5000 ppm (↓32% and ↓48%, respectively for Days 1-8). Mean body weight gains remained low in animals receiving 5000 ppm in the diet for the remaining treatment period. The overall (Day 1-29) body weight gains were statistically significantly decreased in the 2500 and 5000 ppm groups (↓15% and ↓24% respectively) compared to controls.

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The Natural Killer (NK) cells activity was not evaluated in this study. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

For immunotoxicity related to treatment with Tetramethrin, the NOAEL is 5000 ppm (equivalent to 545 mg/kg/day, the highest dose tested). The LOAEL was not determined.

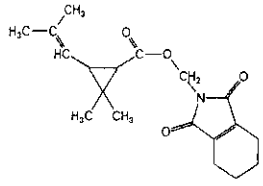
This 28-day dietary immunotoxicity study in the rat is **acceptable/guideline** and satisfies guideline requirement for an immunotoxicity study (OPPTS 870.7800).

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, Quality Assurance and flagging statements were provided. The GLP stated: "These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements". The study was in compliance with GLPs from the UK and OECD.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Tetramethrin

Description:	Crystalline powder
Lot/batch #:	Batch No. 091007
Purity:	95% a.i.
Compound stability:	At least 22 days at ambient temperature (from a previous study, Huntington Life Sciences Study No. VRY0019)
CAS # of TGAI:	7696-12-0
Structure:	

2. Vehicle and/or positive control: The vehicle for the test substance was the diet. Cyclophosphamide (Acros, Batch number: A0277203) was the positive control).

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley (Crl:CD)	
Age/weight at study initiation:	44 to 51 days / 166 to 216 g. grams for females	
Source:	Charles River (UK) Limited	
Housing:	Two per polycarbonate cage with stainless steel mesh lid	
Diet:	Rat and Mouse No.1 Maintenance Diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	40-70%
	Air changes:	not given
	Photoperiod:	12 hrs dark/ 12 hrs light;
Acclimation period:	8-9 days	

B. STUDY DESIGN:

1. In life dates:
Start: January 6-7, 2011 (initiation of treatment)
End: February 3-4, 2011 (termination)

2. Animal assignment: Animals were randomly assigned to the test groups shown in Table 1. According to the study protocol, animals were assigned to the study by one animal at a time was placed in each cage with the procedure being repeated until each cage held the appropriate number of animals. Females were used as they were the most sensitive sex.

Table 1. Study design [a]			
Group	Concentration in Diet (ppm)	Actual Dose to animal (mg/kg/day)	Number of Animals Females
1 Control (vehicle)	0	0	10
2 Low-dose	1000	102	10
3 Mid-dose	2500	257	10
4 High-dose	5000	545	10
5 Positive control (cyclophosphamide)[b]	--	50[b]	8

[a] Data were obtained from pages 9 and 13 of the study report.

[b] Dosed with an single intraperitoneal injection of 50 mg/kg cyclophosphamide at a dose volume of 10 mL/kg (5 mg/mL) two days prior to scheduled sacrifice.

3. **Dose selection rationale:** The report stated that the dose levels used in the present study were selected by the Sponsor on the basis of previous studies conducted with the test substance.

4. **Diet preparation and analysis:** The dosing formulations were prepared weekly. The suitability of the proposed mixing procedures was confirmed as part of a separate study (Huntingdon Life Sciences Study No. VRY0019). In that study Rat and Mouse No. 1 Maintenance diets containing Tetramethrin at 1000 or 15,000 ppm were shown to be homogenous and stable for at least 22 days at ambient (nominally +21 °C) or frozen (nominally -20°C) temperatures. Dose preparations were stored at room temperature, protected from light, until dosing. Data was not included in the study report.

   Test substance concentrations were analyzed using a validated high performance liquid chromatography (HPLC) method.

   Results:

   Stability analysis: Stability was determined in Huntingdon Life Sciences Study No. VRY0019. In that study Rat and Mouse No. 1 Maintenance diets containing Tetramethrin at 1000 or 15000 ppm were shown to be homogenous and stable for at least 22 days at ambient (nominally +21 °C) or frozen (nominally -20°C) temperatures. Data was not included in the study report.

   Homogeneity analysis: Mean homogeneity values determined in Huntingdon Life Sciences Study No. VRY0019. In that study Rat and Mouse No. 1 Maintenance diets containing Tetramethrin at 1000 or 15,000 ppm were shown to be homogenous and stable for at least 22 days at ambient (nominally +21 °C) or frozen (nominally -20°C) temperatures. Data was not included in the study report.

   Concentration analysis: At Week 1 of treatment, representative samples (approximately 200 g) of test diet were taken from the Turbula mixer drum by Pharmacy personnel and submitted for analysis. Each diet sample was sub-sampled (10g) in duplicate and analyzed in accordance with the analytical procedure. The mean concentrations of Tetramethrin in the three test formulations analyzed in Week 1 were in

the range +4.4 to +6.0% of the nominal concentration. All concentration verification results were acceptable.

5. **Statistics:** The following statistical methods were used to evaluate the data for body weight, body weight change, food and water consumption, organ weights and PFC assay.

"A parametric analysis was performed if Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level. The F_1 approximate test was applied. This test is designed to detect significant departure from monotonicity of means when the main test for the comparison of the means is a parametric monotonic trend test, such as Williams' test (Williams 1971, 1972). The test statistic compares the mean square, NMS, for the deviations of the observed means from the maximum likelihood means, calculated under a constraint of monotonicity with the usual error mean square, EMS. The null hypothesis is that the true means are monotonically ordered. The test statistic is F_1 = NMS/EMS which can be compared with standard tables of the F distribution with 1 and EMS degrees of freedom. If the F_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F_1 approximate test was significant, suggesting that the dose response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead."

"A non-parametric analysis was performed if Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. The H_1 approximate test, the non-parametric equivalent of the F_1 test described above, was applied. This test is designed to be used when the main test for comparison of the means is a non-parametric monotonic trend test, such as Shirley's test (Shirley 1977). The test statistic compares the non-monotonicity sums of squares, NRSS, for the deviations of the observed mean ranks from the maximum likelihood mean ranks with the non-parametric equivalent of the error sums of squares, ERSS = N(N+ 1)/12. The test statistic is H_1 = NRSSIERSS which can be compared to standard tables of the χ^2-distribution with 1-degree of freedom. If the H_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H_1 approximate test was significant suggesting that the dose-response was not monotone, Steel's test. (Steel 1959) was performed instead."

C. METHODS:

1. **Clinical Signs:** Animals were inspected visually at least twice daily for evidence of changes in health or reaction to treatment. Cages were inspected daily for evidence of ill-health amongst the animals. Deviations from normal were recorded at the time of observation as to the nature and severity, date and time of onset, duration and progress, as appropriate.

   Detailed weekly physical examinations were performed to monitor general health.

2. **Body weight:** Body weights were recorded twice during the pre-dose phase, before dosing on Day 1, and then biweekly during dose administration, and at the time of sacrifice. Positive controls animals were weighed on Day 27, at administration of cyclophosphamide.

3. **Food consumption and test substance intake:** Food consumption was determined weekly,

by cage, during dose administration. The weight of the food supplied to each cage, the remains of food after feeding + an estimate of amount spilled weekly was recorded. Food consumption was calculated by each cage as (g/animal/week).

Test substance uptake was determined by:

$$\text{Achieved max dose (as mg/kg/day)} = \frac{\text{Food consumption (g/animal) x ppm test substance}}{\text{Mid-week body weight (g) x 7}}$$

4. **Water consumption:** Water consumption was determined weekly by recording weight of water consumed over a 3-day period for each week.

5. **Sacrifice and pathology:** After 4 weeks of treatment, animals were sacrificed by carbon dioxide asphyxiation, followed by exsanguination. Terminal body weights were recorded. The necropsy included examination of external carcass features; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues. The adrenals, spleen and thymus from each animal were weighed. Organ-to-terminal body weight ratios were calculated. Adrenals, lymph nodes (mandibular, mesenteric, and left axillary), Peyer's patches, and thymus samples (or the whole) of the other tissues from all animals were preserved in 10% neutral buffered formalin. The whole spleen was transferred to individual containers of Hank's Balanced Salt Solution (HBSS) and held on ice (water) until processed for analysis. Splenocyte suspensions were prepared by mechanical dissociation and used for the plaque forming cell (PFC) assays.

6. **Antibody plaque-forming cell (PFC) assay:** Four days prior to scheduled sacrifice (Day 25), all animal were immunized with an intravenous (1.0 mL bolus injection) of 2×10^8 sheep red blood cells (SRBC)/animal via the tail vein.

   On day 29, animals were sacrificed and their spleens were removed for the plaque-forming cell (PFC) assays. Single-cell suspensions of each spleen were prepared by mechanical dissociation and used for the plaque forming cell (PFC) assays. Total cellularity and viability were determined for each sample. Splenocytes were mixed with fresh SRBC and guinea pig complement in a semisolid agarose matrix. Viability testing of mononuclear cells was conducted on spleen cell preparations using a Trypan blue dye exclusion method. The cell counts were used to calculate the number of viable spleen cells/mL, excluding red blood cells.

   The group mean numbers of mononuclear cells/spleen were calculated and the numbers of plaques were determined and expressed as PFC/10^6 spleen cells and PFC/spleen.

7. **Natural Killer (NK) cells activity assay**: NK cells activity assay was not performed in this study.

II. RESULTS:

A. OBSERVATIONS:

1. **Mortality:** There were no treatment-related deaths during the course of the study.

2. **Clinical signs of toxicity:** There were no significant test substance-related clinical observations.

B. BODY WEIGHT AND WEIGHT GAIN:

There was a statistically significant reduction of body weight gain in females receiving 2500 or 5000 ppm (↓32% and ↓48%, respectively for Days 1-8), with weight gain remaining low for the rest of the treatment period in those receiving 5000 ppm. The overall (Day 1 to 29) bodyweight gains at 2500 and 5000 ppm were decreased significantly (about ↓15% and ↓24% respectively), than controls. Mean body weights and mean body weight gains are presented in Table 2.

Table 2. Mean body weights and body weight gains during the treatment period [a]

Tetramethrin in diet (ppm)	Mean body weight (grams±SD) (n=10)						Mean body weight gain		
	Day 1	Day 4	Day 8	Day 15	Day 22	Day 29	Day 1-8	Day 7-29	Day 1-29
0	191±9.3	205±4.8	216±6.2	233±12.1	244±13.2	261±13.6	24±5.0	45±9.0	69±9.3
1000	191±14.7	203±16.4	214±17.0	231±19.5	244±19.9	260±21.0	24±7.9	45±8.6	69±14.8
2500	191±7.8	200±13.0	208±13.2	224±15.9	233±12.9	250±14.7	16*±8.0 (↓32)	42±6.2	58*±10 (↓15)
5000	188±10.9	193±13.7	201±13.1	217±15.7	225±17.7	240±19.4	13**±4.2 (↓48)	40±7.6	52*±9.7 (↓24)

[a] Data were obtained from Table 2 page 33-34 of the study report. (↓↑ percent change calculated by reviewer.)
* Significantly different from the control ($p \leq 0.05$).
** Significantly different from the control ($p \leq 0.01$).

C. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE:

1. **Food consumption:** There was no statistically significant treatment-related changes in food consumption. Mean food consumption during the treatment period were slightly decreased in all treatment groups Days 1-7 and slightly increased in all treatment groups Days 8-14 when compared to the control. Food consumption data is summarized in Table 3.

Table 3. Mean food consumption during the treatment period [a]

Tetramethrin in diet (ppm)	Mean food consumption (grams/animal/week±SD) (n = 10)					
	Days -7-0	Days 1-7	Days 8-14	Days 15-21	Days 22-28	Days 1-28
0	130±7.2	155±12.0	167±15.9	167±12.5	174±7.1	166±9.5
1000	126±11.9	147±13.0	171±20.4	160±16.4	172±14.7	162±13.0
2500	127±5.6	143±7.7	185±26.5	152±8.2	155±7.1	159±7.1
5000	135±2.9	146±8.5	186±12.8	152±16.4	168±16.3	163±10.7

[a] Data were obtained from Table 2, page 35 of the study report.

2. **Test substance consumption:** The overall mean consumption are summarized in Table 1.

3. **Water consumption:** Overall group mean water consumption was not significantly different from the control value.

D. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related macroscopic findings.

2. **Organ weights:** There were statistically significant treatment-related effects on mean terminal body weights but not on selected organ weights.

   Mean terminal body weights and mean absolute and relative to terminal body weights for adrenals, spleen, and thymus are summarized in Table 4.

Table 4. Mean organ weights (g) and mean organ/ body weight ratios (%) [a]

Tetramethrin in diet (ppm)	Terminal body weight (grams ±SD)	Adrenals		Spleen		Thymus	
		Absolute weight (grams±SD)	Relative weight (%)	Absolute weight (grams±SD)	Relative weight (%)	Absolute weight (grams±SD)	Relative weight (%)
0	260±13	0.069±0.013	0.0267±0.0044	0.616±0.090	0.237±0.036	0.415±0.063	0.160±0.026
1000	258±21	0.069±0.010	0.0268±0.0034	0.618±0.103	0.241±0.048	0.402±0.077	0.156±0.027
2500	248±15	0.065±0.006	0.0262±0.0018	0.539±0.068	0.218±0.025	0.357±0.075	0.144±0.029
5000	238**±19 (↓8.5)	0.070±0.010	0.0294±0.0044	0.588±0.075	0.247±0.025	0.347±0.116	0.145±0.042

[a] Data were obtained from Tables 5, pages 37-38 of the study report. (↓↑ percent change calculated by reviewer.)
** Significantly different from the control ($p \leq 0.01$).
Treated groups compared to control using Williams' test.

3. **Histopathology:** Histopathology was not performed in this study.

E. ANTIBODY PLAQUE-FORMING CELL (PFC) ASSAY:

As determined by the PFC assay, there was no statistically significant effect observed on the number of antibody secreting cells, expressed as either total activity (PFC/spleen) or specific activity (PFC/10^6 spleen cells) at 1000, 2500, and 5000 ppm in the diet (Table 5). High inter-individual variability was seen in the treatment and vehicle control groups. Examination of individual animal data did not show any trend or distribution that would demonstrate significant suppression of the anti-SRBC immune function parameters (Figures 1 and 2). The positive control (cyclophosphamide) group showed statistically significant decreases in immune function parameters (PFC/spleen, PFC/10^6 splenocytes, and absolute splenocyte viability, confirming the validity of the immunotoxicity assay.

Table 5. Antibody plaque-forming cell (PFC) assay (mean±SD) [a]

Concentration in (ppm)	Number of animals	Viable cells/spleen (x 10^7)	PFC/10^6 viable cells	PFC/spleen
0	10	28.0±9.58	767±417	203790±104966
1000	10	27.5±9.06	710±499	204694±189576
2500	10	19.6±6.13	474±260	98783±77075
5000	10	29.4±8.99	545±304	171144±124257
Positive control - cyclophosphamide	8	9.45^{***L}±3.22 (↓66)	21.3^{***S}±20.5 (↓97)	1651^{+++}±1335 (↓99)

[a] Data were obtained from page 27 and Annex 2, Table 1, page 110 of the study report.
(↓↑ percent change calculated by reviewer.)
L data log transformed prior to analysis, observed means presented.
S data square-root transformed prior to analysis, observed means presented.
*** $p<0.001$ for comparisons with Group 1 using t test.
+++ $p<0.001$ for comparisons with Group 1 using Wilcoxon rank sum test.
PFC = Plaque forming cells

Figure 1 Plaque forming cell assay (PFC/10^6 viable cells) - females

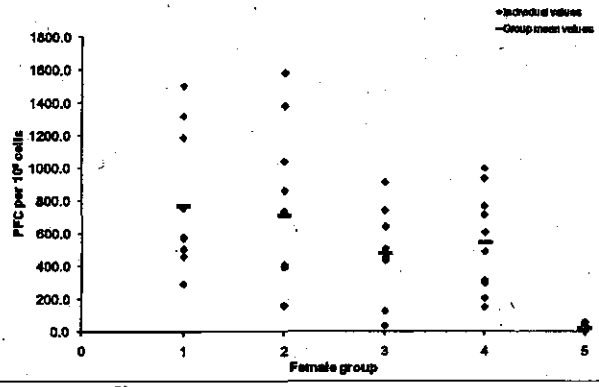
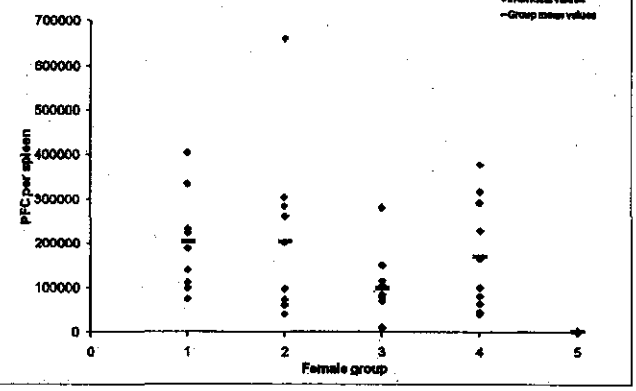


Figure 2 Plaque forming cell assay (PFC/spleen) - females



Key:
Group 1 = Control, 0 ppm
Group 2 = Tetramethrin, 1000 ppm
Group 3 = Tetramethrin, 2500 ppm
Group 4 = Tetramethrin, 5000 ppm
Group 5 = Cyclophosphamide, 50 mg/kg

F. NATURAL KILLER (NK) CELL ACTIVITY ASSAY: NK cells activity assay was not performed in this study.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that "dietary administration of Tetramethrin to female rats at dietary concentrations of 1000, 2500 or 5000 ppm for four weeks caused a non-specific toxic response at 2500 and 5000 ppm. Findings at 2500 ppm were limited to a reduction in bodyweight gain in Week 1 whilst at 5000 ppm there was a reduction in body weight gain, predominantly in Week 1. There was no effect on the immune function, as assessed by the measurement of antigen-specific, T-cell dependent antibody formation. The no-observed adverse-effect level (NOAEL) for immunotoxicity of Tetramethrin was therefore greater than 5000 ppm in females (i.e. >545 mg/kg/day)."

B. REVIEWER COMMENTS: In an immunotoxicity study (MRID 48540401), Tetramethrin was administered in the diet to female Sprague-Dawley rats (10/group) at concentrations of 0, 1000, 2500, or 5000 ppm (equivalent to 102, 257 or 545 mg/kg/day respectively) for 28 consecutive days. Animals in the positive control group (8/group) received a single intraperitoneal injection of cyclophosphamide (50 mg/kg) at a dose volume of 10 mL/kg (5 mg/mL) on Day 27. There were no treatment-related effects on mortality, clinical observations, food or water consumption, mean absolute and relative (to terminal body weight) organ (adrenals, spleen, or thymus) weights in any group.

There was a statistically significant reduction of body weight gain in females receiving 2500 or 5000 ppm (↓32% and ↓48%, respectively for Days 1-8). Mean body weight gains remained low in animals receiving 2500 and 5000 ppm in the diet for the remaining treatment period. The overall (Day 1-29) body weight gains were statistically significantly decreased in the 2500 and 5000 ppm groups (↓15% and ↓24% respectively) compared to controls.

For systemic toxicity related to treatment with Tetramethrin, the LOAEL was 2500 ppm; based on overall decreased mean body weights gains. The NOAEL for systemic toxicity in female Sprague-Dawley rats is 1000 ppm.

Evaluation of immune function using an antibody plaque-forming cell (PFC) assay indicated that Tetramethrin had no treatment-related effect on PFC/10^6 cells, PFC/spleen, and cells/spleen or spleen and thymus weights. High inter-individual variability was seen in the treatment and vehicle control groups. Examination of individual animal data did not show any distribution that would demonstrate significant suppression of the anti-SRBC immune function parameters. The positive control group showed statistically significant decreases in immune function parameters (absolute splenocyte viability, PFC/10^6 splenocytes, and PFC/spleen at ↓66, ↓97 and ↓99%, respectively), confirming the validity of the immunotoxicity assay.

The Natural Killer (NK) cells activity was not evaluated in this study. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

For immunotoxicity related to treatment with Tetramethrin, the NOAEL is 5000 ppm (equivalent to 545 mg/kg/day). The LOAEL was not determined.

C. STUDY DEFICIENCIES:

There were no major study deficiencies. Minor deficiencies were as follows:

Body weights, body weight gains, food and water consumption, mean absolute and relative organ (adrenals, spleen and thymus) weights were not reported for the positive control group.

Stability and homogeneity were cited from another report, but data was not included in the study report.